

COTYLEDON AREOLES IN THE FABACEAE SUBTRIBE CLITORIINAE

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ABSTRACT

Of the five genera of Phaseoleae subtribe Clitoriinae, four genera; *Centrosema*, *Periandra*, *Clitoria*, and *Barbieria*; were sampled for cotyledon areole presence or absence, and for other obvious anatomical characters in mature dormant seeds. *Clitoriopsis* seeds were unavailable. All specimens examined have cotyledon areoles except in *Clitoria* subgenus *Bractearia* and two species of *Clitoria* subgenus *Neurocarpum*. Most cotyledon areoles are small, circular to ovate, and approximately medial, and their presence or absence, position, and size are directly related to endosperm occurrence. The limited sampling indicates that the subtribe has diverse seed anatomy, evident by several distinctive characters in various combinations. *Centrosema* and *Periandra* have only spongy cotyledon mesophyll, a peculiar lens structure of tracheoids, and a dorsal tracheid bar extension. *Barbieria* has two hilar tongues, palisade mesophyll, and a viscid, clear, colorless, and thermoplastic epitesta. Of the three subgenera of *Clitoria*, subgenus *Clitoria* has palisade mesophyll; subgenus *Bractearia* has only spongy mesophyll and two hilar tongues; and subgenus *Neurocarpum* has only spongy mesophyll, two hilar tongues, and the same epitesta as *Barbieria*.

KEY WORDS: Cotyledon areoles, endosperm, galactomannan

RESUMEN

De los cinco géneros de Phaseoleae subtribu Clitoriinae, cuatro; *Centrosema*, *Periandra*, *Clitoria*, y *Barbieria*; se muestrearon para la presencia o ausencia de areola en el cotiledón, y otros caracteres anatómicos en las semillas durmientes. Las semillas de *Clitoriopsis* no estuvieron disponibles. Todos los especímenes examinados tienen areolas en el cotiledón excepto *Clitoria* subgénero *Bractearia* y dos especies de *Clitoria* subgénero *Neurocarpum*. La mayoría de las areolas del cotiledón son pequeñas, de circulares a ovadas, y aproximadamente mediales, y su presencia o ausencia, posición, y tamaño están directamente relacionados con la presencia de endospermo. El muestreo limitado indica que la subtribu tiene una anatomía seminal diversa, evidenciada por varios caracteres distintivos en varias combinaciones. *Centrosema* y *Periandra* tienen sólo mesófilo cotilar esponjoso, una peculiar estructura lenticular de traqueoides, y una extensión dorsal de una barra de traqueidas. *Barbieria* tiene dos lenguas hilares, mesófilo en empalizada, y una epitesta viscosa, clara, sin color, y termoplástica. De los tres subgéneros de *Clitoria*, el subgénero *Clitoria* tiene mesófilo en empalmezado; el subgénero *Bractearia* tiene únicamente mesófilo esponjoso y dos lenguas hilares; y el subgénero *Neurocarpum* tiene únicamente mesófilo esponjoso, dos lenguas hilares, y la misma epitesta *Barbieria*.

INTRODUCTION

The cotyledon areole, named by Endo and Ohashi (1997, 1998a, 1998b, 1999a, 1999b), is a small spot of projecting epidermal cells on the abaxial cotyledon surface of dormant mature seeds of many Leguminosae subfamily Papilionoideae, but absent in subfamilies Caesalpinioideae and Mimosoideae. Cotyledon areole cells, and subtending cells, have different size, shape, and several peculiar anatomical and chemical characteristics compared with surrounding cells. The presence or absence, shape, and position seem to mark certain genera, tribes, and groups of tribes. In the Clitoriinae, Endo and Ohashi (1999a) found medial cotyledon areoles in one unidentified species each of *Clitoria* and *Centrosema*. They established that the soybean structure called a “pit” (Dzikowski 1936, 1937; Miksche 1961; Yaklich et al. 1984, 1986, 1987, 1989, 1992, 1995, 1996, 1998; Baker & Minor 1987; Ma et al. 2004) is the same as a cotyledon areole. Beck’s (1878) “Aleuronfleck,” described and illustrated in detail, also matches the cotyledon areole. Cotyledon areole function is unknown (Ma et al. 2004).

Despite incidental references to Clitoriinae seed anatomy as part of larger projects, studies of specific features, or reviews at a generic level (Pammel 1899; Martin 1946; Lersten 1981, 1982; Kirkbride et al. 2003), there is no basic anatomical study of these seeds.

Because cotyledon areole presence or absence, shape, and position seemed to have taxonomic significance, because of the lack of basic comparative seed anatomy in Clitoriinae, and because cotyledon areoles have not been studied in relation to other features of these seeds, the current study has the following aims: 1) to survey for cotyledon areole presence in the subtribe; 2) to study basic seed anatomy to determine if cotyledon areoles are correlated with other seed features; and 3) to evaluate if any of these characters could yield data of systematic significance.

MATERIALS AND METHODS

Studied seeds were deposited at BARC or US (Table 1). All specimens were assigned a unique number, preceded by the letters JAL. Plant names and authors follow treatments given by Fantz cited in the Discussion. In *Clitoria* species, the subgenus name is indicated in parentheses.

Seeds were examined dry, both externally and internally, with a Wild M5 stereomicroscope. Seeds were examined whole or cracked longitudinally or transversely with a razor blade and a custom-made cutting block or with a miniature angled end cutter (Tronex 5083, reground to zero microbevel). For enhanced visibility, a drop of toluidine blue O solution, 0.05% in 1M phosphate buffer pH 6.76, was sometimes applied to the abaxial surface of dry cotyledons to color most epidermal cells more strongly than the more lightly staining cotyledon areole cells. Sketches of seeds were made with a camera lucida microscope attachment. Line work was done in Adobe Illustrator. Conventions for distinguishing features by stippling and other patterns were adapted from those of Schleiden and Vogel (1839), Corner (1951), and Smith (1981, 1983). Views within each drawn sample may have come from different seeds, and thus may differ in size and shape. Photographs were taken with a digital camera on a dissecting or compound microscope, and sometimes processed with extended depth of field software.

Orientation terms follow the definitions of Sterling (1954). Median plane is the plane passing through the hilar groove, micropyle, and chalaza and perpendicular to the hilum surface. Transverse plane is any plane perpendicular both to the median plane and to the hilum surface. Frontal plane is any plane parallel to the hilum surface, and therefore perpendicular both to the median and transverse planes. Anterior is towards the micropyle end of the seed. Posterior is towards the lens end of the seed. Dorsal is the side of the seed farthest from the hilum. Ventral is at the side of the seed nearest the hilum. Lateral is at the surface of the seed on either side of the median plane. Right and left are from the point of view of an observer oriented as the seed is oriented, with top to the anterior, bottom to the posterior, dorsal to the back, and ventral to the front. Pod orientation conventions for ventral, dorsal, anterior, posterior, right, and left, are the same as for the seed, with anterior towards the stigma, posterior to the base, ventral towards the suture bearing the ovules, and dorsal towards the opposite suture.

Seed terminology and abbreviations were adapted from Schleiden and Vogel (1839), Corner (1951), Gunn (1981), and Kirkbride et al. (2003): **al** = aleurone layer (outer endosperm layer), **ar** = aril (strophiole and caruncle of some authors), **arp** = antiraphe, **ca** = cotyledon areole, **cl** = crushed endosperm layer (inner endosperm layer, third layer), **cpal** = counter palisade, **ch** = chalaza, **cot** = cotyledon, **cu** = cuticle, **em** = embryo, **en** = endosperm, **eph** = epihilum, **ept** = epitesta, **f** = funiculus, **h** = hilum, **hgl** = hourglass cells, **hgr** = hilar groove, **ht** = hilar tongue, **l** = lens (strophiole of some authors), **ll** = light line, **m** = micropyle, **ml** = middle endosperm layer (swelling layer), **pal** = palisade (Malpighian) cells, **p** = plumule, **pm** = palisade mesophyll, **r** = radicle, **ra** = rim-aril, **rp** = raphe, **r vb** = recurrent vascular bundle, **tb** = tracheid bar, and **vb** = vascular bundle. Format for seed descriptions generally follow Kirkbride et al. (2003). Descriptive terms for cotyledon areole position follow Endo and Ohashi (1999a); the cotyledon is divided into five equal parts from the base to apex: basal, basal-medial, medial, medial-apical, apical.

Presence of starch was tested by depositing one drop of Lugol's iodine solution (iodine 0.2 g, potassium iodide 0.3 g, water 30 ml) on the cotyledon surface cut in cross section. Starch grains produced a purple to black color within seconds, when observed under the dissecting microscope. Hydration of seeds was aided with Pohlstoff (Aerosol OT 1 ml, methanol 25 ml, water 74 ml).

Uncoated seed material was observed with a Philips XL30 ESEM.

TABLE 1. Specimens studied.

JAL	Species	Specimen
1261	<i>Barbieria pinnata</i> (Persoon) Baill.	Misc. US (BARC)
1271	<i>Barbieria pinnata</i> (Persoon) Baill.	Sinteris 5942 (BARC) Puerto Rico
1262	<i>Centrosema arenarium</i> Benth.	IRI 1355 (BARC) Brazil
1267	<i>Centrosema brasilianum</i> (L.) Benth.	Rodrigues 643 (US) Brazil
1268	<i>Centrosema paseuouiium</i> Mart.	Pittier 13474 (US) Venezuela
200	<i>Centrosema plumeri</i> (Turp.) Benth.	PI 247478 (BARC) Belgian Congo
202	<i>Centrosema plumeri</i> (Turp.) Benth.	PI 322329 (BARC) Brazil
1266	<i>Centrosema plumeri</i> (Turp.) Benth.	Nee 9492 (BARC) Panama
208	<i>Centrosema pubescens</i> Benth.	PI 286289 (BARC) Ivory Coast
209	<i>Centrosema pubescens</i> Benth.	PI 279594 (BARC)
210	<i>Centrosema pubescens</i> Benth.	PI 219833 (BARC) Ceylon
211	<i>Centrosema pubescens</i> Benth.	PI 212980 (BARC) India
1015	<i>Centrosema pubescens</i> Benth.	Pollard et Palmer 328 (BARC) Cuba
1264	<i>Centrosema pubescens</i> Benth.	PI 308555 (BARC)
1265	<i>Centrosema pubescens</i> Benth.	PI 337079 (BARC)
282	<i>Centrosema sagittatum</i> (Willd.) Malme	Pederson 2796 (BARC) Argentina-MO
500	<i>Centrosema sagittatum</i> (Willd.) Malme	Pederson 2796 (BARC) Argentina-K
1263	<i>Centrosema sagittatum</i> (Willd.) Malme	Shafer 481 (US) Cuba
204	<i>Centrosema</i> sp.	PI 200731 (BARC)
203	<i>Centrosema virginianum</i> (L.) Benth.	PI 322356 (BARC) Brazil
205	<i>Centrosema virginianum</i> (L.) Benth.	PI 322351 (BARC) Brazil
224	<i>Centrosema virginianum</i> (L.) Benth.	55-17 Urbana (BARC)
1190	<i>Centrosema virginianum</i> (L.) Benth.	Wolff 3138 (US) Texas
1594	<i>Clitoria amazonum</i> Mart. Ex Benth.	Martinelli 7263 (US) Brazil
1595	<i>Clitoria brachycalyx</i> Harms	Hahn 5612 (US) Guyana
296	<i>Clitoria laurifolia</i> Poir.	CPI 34890 CSIRO (BARC)
1599	<i>Clitoria mariana</i> L.	Bain s.n. (US) Tennessee
295	<i>Clitoria rubiginosa</i> Pers.	CPI 35685 CSIRO (BARC)
197	<i>Clitoria ternatea</i> L.	PI 209315 (BARC)
1270	<i>Periandra densiflora</i> Benth.	Pires 58051 (US) Brazil
252	<i>Periandra heterophylla</i> Benth.	PI 322570 (BARC)
501	<i>Periandra heterophylla</i> Benth.	Philcox et Ferreira 3874 (BARC) Brazil
1269	<i>Periandra heterophylla</i> Benth.	IRI 1779 (BARC) Brazil
1592	<i>Periandra heterophylla</i> Benth.	Irwin et al. 15211 (US) Brazil
1593	<i>Periandra mediterranea</i> (Vell.) Taub.	Irwin et al. 15430 (US) Brazil
1596	<i>Periandra mediterranea</i> (Vell.) Taub.	Irwin et al. 7969 (US) Brazil

RESULTS

Because seed morphology in the Clitoriinae is diverse, four detailed descriptions are given for *Centrosema virginianum*, *Clitoria* (*Clitoria*) *ternatea*, *Clitoria* (*Neurocarpum*) *rubiginosa*, and *Barbieria pinnata*. Other species generally correspond to one of these four detailed descriptions, and will thus be described in notes and differential diagnoses. Results are summarized (Table 2). Size variation from specimen to specimen and seed to seed is common. Measurements should be regarded as a general guide and not a comprehensive indication of variation for the species. Specimens of several species were inadequate in quantity and quality.

Centrosema virginianum. Seed reniform-oval to cylindrical; ca 4.3×1.5×2.5 mm (length×width×height) (Fig. 1a); brown with brown hilum; sink in water; micropyle oriented towards stigma in pod.

Seed externally with prominent hilum, anterior micropyle and posterior lens (Fig. 2e). Hilum oval ca 1.3×0.7 mm; surrounded by a raised rim. Rim-aril brown, mostly of ca 0.05 mm raised bead; with hilar tongue ca 0.7 mm wide by 0.1 mm long positioned from mid-hilum extending toward anterior end. Hilar tongue on left in about half the seeds; on right in about half the seeds. Counter-palisade surface naked or

TABLE 2. Summary of seed characters.

Taxon	CA	Lens	TB	Hilar tongue	Epitesta	Cotyledon	Germ	Endosperm	Starch	Can	Distribution	Spp
Centrosema	M	Tracheoids	Wing	One	-	Spongy	H / E	ML	-	Can	Americas	36
Periandra	M	Tracheoids	Wing	One	-	Spongy	E / G	ML	-/Starch	-	Brazil, Bolivia	6
Clitoria subg. Clitoria	M	-	-	One	-	Palisade	E	ML	-	-	Old World?	9
Clitoria subg. Bractearia	-	-	-	Two	-	Spongy	E	-	Starch	-	S & C America, Mexico	29
Clitoria subg. Neurocarpum	-(BM)	-	-	Two(-One)	Viscid	Spongy	H (E)	-(ML)	Starch (-)	-	Americas?, Asia	24
Barbieria	BM	-	-	Two	Viscid	Palisade		ML	-		S & C America, Mexico	1

Results from current work and previous literature. Germination is from Baudet 1974; Fantz 1979a, 1988; Holm 1891; Pammel 1899; Yeh et al. 1987, with terms from Duke (1981). Canavanine presence is from Lackey 1977a. Distribution and species counts are from Fantz 1979a, 1988; Schrire 2005. Blanks for character state indicate no data available; + = present, - = absent, ? = observation is uncertain, a/b = multiple states, a-b = intermediate state; () = state for *Clitoria mariana*. CA, cotyledon areole: M = medial, BM = basal-medial; Lens: lens cells: Tracheoids = pitted tracheoids present; TB, tracheid bar: Wing = dorsal wing present; Hilar tongue: hilar tongue number; Epitesta: epitesta present or absent, if present, Viscid = viscid epitesta; Cotyledon, cotyledon mesophyll: Spongy = spongy throughout, but sometimes with some slight palisade orientation, Palisade = palisade tissue adaxially and abaxially; Germ, germination: H = hypogeal, E = epigeal, G = geal; Endosperm, middle endosperm layer apparent: ML = middle layer apparent; Starch, cotyledon starch test positive or negative: Starch = positive; Can, canavanine in embryo: Can = positive; Distribution: general distribution; Spp: number of species.

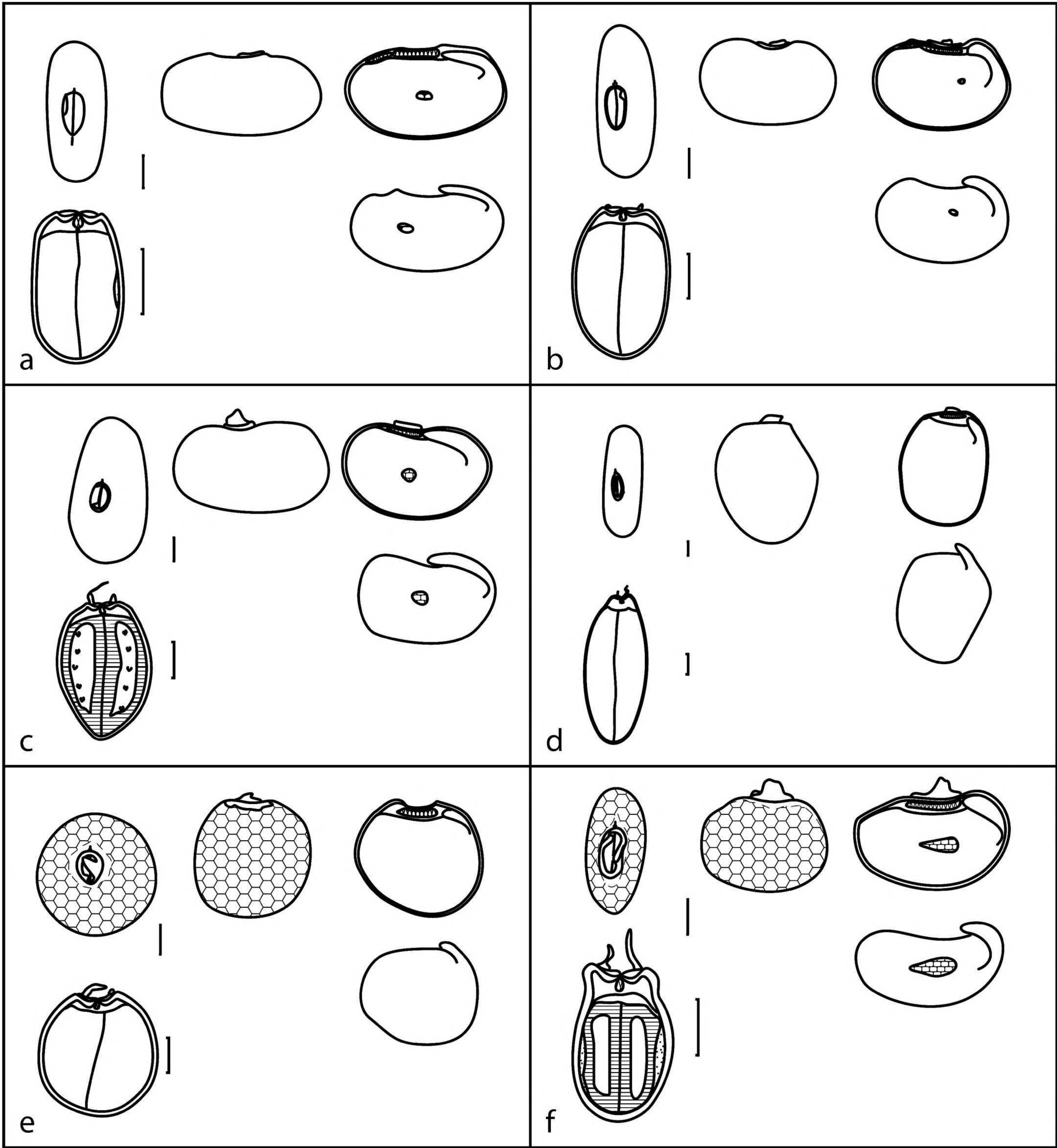


FIG. 1. Camera lucida drawings of Clitoriinae seeds. Each figure is composed of the following views: in upper left is ventral view, anterior towards top of page, of exterior of seed showing hilum. In upper center is lateral view of left side of seed, anterior facing right side of page. In upper right is longitudinal section through median plane of seed, anterior to right side of page, showing interior of testa with adherent endosperm lining the embryo cavity, embryo removed. Tracheid bar and any tracheoids of lens on ventral side indicated by crosshatching. Impression of cotyledon areole on endosperm indicated by outline with brick-like pattern. Endosperm cut by longitudinal section shown by stippling. In lower right is left side of embryo. Cotyledon areole indicated by outline with brick-like pattern. In lower left is cross section, with ventral side towards top of page, through middle of hilum. Tracheid bar and lens tracheoids indicated by crosshatching. Endosperm indicated by stippling. Palisade tissue indicated by parallel lines. Spongy mesophyll indicated by no pattern. Cotyledon vascular bundles indicated by dashed outlines. Broken parts or missing parts in all views indicated by dashed lines. Each figure is based on the same sample, but individual views may be from different seeds. Scale bars are all 1 mm. Cross sections are generally larger than the remaining views, and have their own scale bar. a. *Centrosema virginianum* JAL 1190. b. *Periandra heterophylla* JAL 252. c. *Clitoria ternatea* JAL 197. d. *Clitoria amazonum* JAL 1594. e. *Clitoria rubiginosa* JAL 293. f. *Barbieria pinnata* JAL 1271.

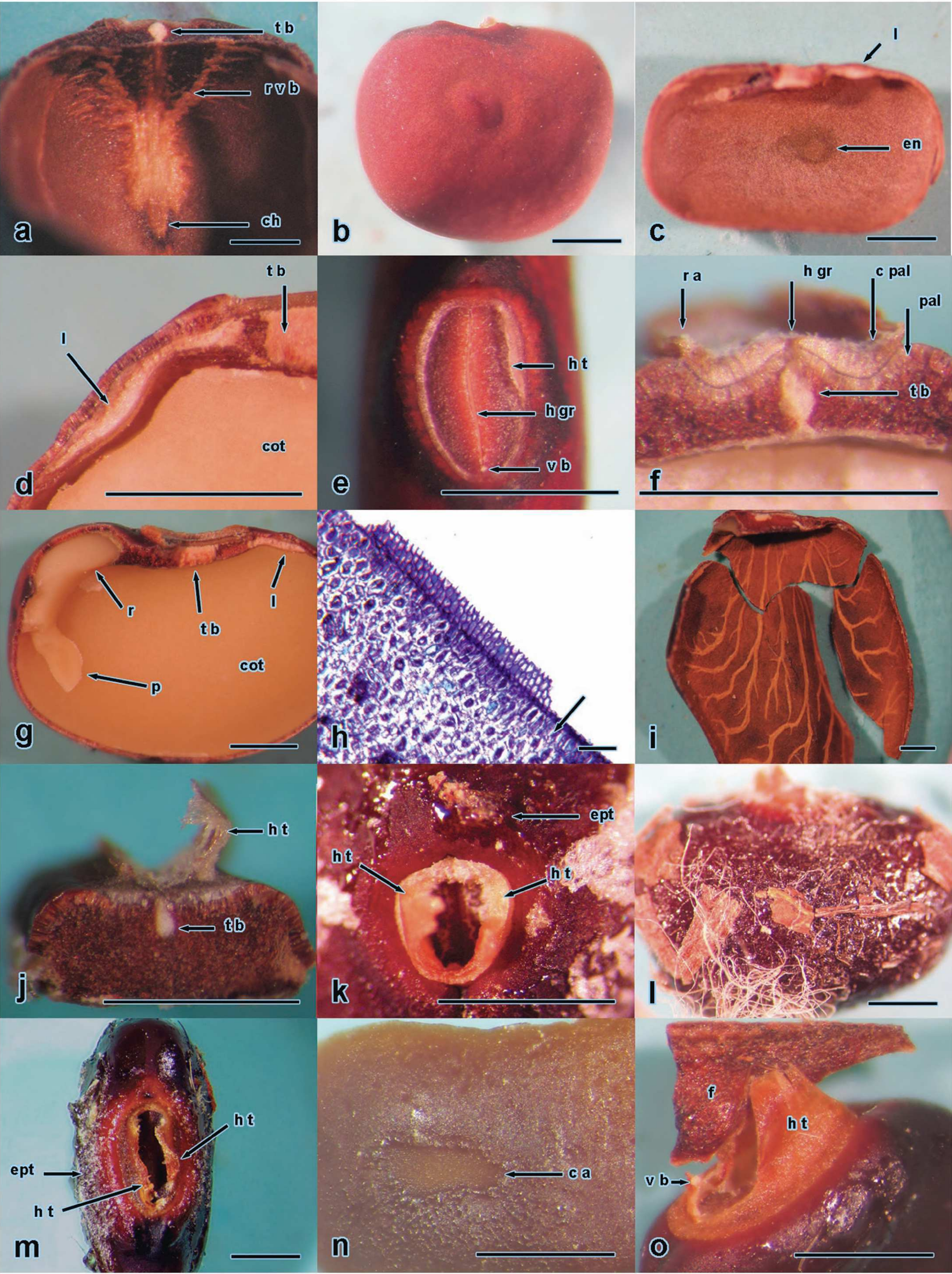


FIG. 2. Photographs under dissecting (a–g, i–o) and compound (h) microscope of Clitoriinae seeds. a. *Centrosema arenarium* JAL 1262. b. *Centrosema brasilianum* JAL 1267. c. *Centrosema pascuorum* JAL 1268. d. *Centrosema* sp. JAL 204. e. *Centrosema virginianum* JAL 1190. f–g. *Periandra heterophylla* JAL 252. h. *Periandra heterophylla* JAL 1269. i–j. *Clitoria amazonum* JAL 1594. k. *Clitoria laurifolia* JAL 296. l–o. *Barbieria pinnata* JAL 1271. Scale bars are 1 mm (a–g, i–o) or 100 μ m (h). a. Transverse section of ventral testa with exposed inner posterior surface. Tracheid bar with extension spans entire

covered with infrequent minute white cellular funicular remnants. Vascular bundle scar between posterior end of hilar groove and rim-aril; white; raised; prominent. Hilar groove visible; when open, allows view of white tracheid bar below; opens and closes in seconds in response to humidity; air bubble often forms on hilum upon immersion in water. Micropyle outside rim-aril, ca 0.05 mm long; Y-shaped when dry, with arms of Y towards hilum. Lens slightly perceptible as ca 0.3 mm cleft; 0.4 mm posterior to hilum. Lateral endosperm deposits sometimes perceptible externally as minute testa swellings. Epitesta deposits wanting.

Seed in transverse section through the middle of the hilum with testa, endosperm, and two cotyledons of the embryo. Testa 0.05 mm thick dorsally to 0.3 mm thick at the hilum. Palisade (Malpighian) layer of one cell-layer thick; thinner dorsally; light line immediately under the outer surface except at the hilum where it shifts inward. Hourglass layer immediately interior; of a single cell layer; imperceptible dorsally, thin laterally, thicker ventrally, disappearing under the hilum. Parenchyma layer imperceptible dorsally and ventrally with 50× optics. Hilum testa much thicker and more differentiated than dorsal and lateral testa. Rim-aril and extension into hilar tongue top counter-palisade at outside edge. Funicular cell remnants few; ventral to counter-palisade. Hilum palisade and counter-palisade curved upwards towards hilar groove. Light line in hilum palisade about one-tenth down from outer surface; touching each other at the lips of the hilar groove when groove closed. Tracheid bar white; ovate; immediately below hilar groove; sometimes minutely cleft ventrally; attenuated dorsally into a contiguous or almost separate wing which touches or nearly reaches the endosperm. Parenchyma below hilum of columns of loosely joined dark cells with large air spaces. Endosperm dorsally and ventrally thin; laterally forming two thick pockets 0.05×0.5 mm, about equal to the thickness of the testa, aleurone layer imperceptible, middle and inner layers tan. Cotyledons off-white; concave on part of abaxial face to match expanded endosperm; mesophyll of spongy parenchyma, some palisade orientation of adaxial cells; starch test negative; vascular bundles 6 or 7 per cotyledon, middle one larger.

Seed in longitudinal section reveals the same basic testa, endosperm, and embryo structure and description will not be repeated from the transverse section unless there is additional information or greater clarity in this view. Testa palisade tissue slightly thicker above radicle than elsewhere. Vascular bundle from funiculus joins posterior end of the tracheid bar and extends into the raphe vascular bundle. Lens tissue between raphe vascular bundle and Malpighian layer of periclinal and scalariform-thickened white tracheoids similar to anticlinal cells of tracheid bar. Endosperm adnate to and lines the entire inner surface of the testa; transparent and almost colorless or slightly tan when uncut, allowing view of inner surface of testa through endosperm, white where cut or broken; when dry forms an impression of every feature of the embryo, down to the cellular level; thicker areas especially between radicle and cotyledon, and in a lateral thickening both left and right; cotyledon areole impression from embryo on each lateral endosperm thickening; swells on addition of water, especially hot water, becomes gelatinous, and embryo impression structure lost.

ventral testa thickness. Recurrent vascular bundles and raphe vascular bundle terminating near the chalaza can be seen through inner testa surface. **b.** Lateral exterior surface of testa, showing bulge at site of endosperm enlargement. **c.** Interior testa surface, showing impression of cotyledon areole into thickened endosperm (marked en) in medial to slightly medial-apical position. **d.** Longitudinal section of ventral posterior part of seed, showing white tracheoid lens tissue. **e.** Ventral exterior view of hilum, showing the usual hilar structures: a single hilar tongue, rim-aril, and funicular vascular bundle scar just posterior to the hilar groove, which is slightly open, allowing view of the white tracheid bar below. **f.** Transverse section of ventral testa and cotyledons, showing tracheid bar, with extension, spanning entire ventral testa thickness. **g.** Longitudinal section of seed, right cotyledon removed. Embryo axis exposed. Posterior portion of tracheid bar and lens visible. **h.** Hand transverse section of adaxial cotyledon tissue stained with toluidine blue. A flap of epidermal tissue with surface cell patterns shows to right in face view. One or two rows of adaxial mesophyll cells are somewhat palisade-like and indicated by arrow. **i.** Reconstructed interior of testa, showing prominent venation from recurrent vascular bundles. **j.** Transverse section of ventral testa, showing the usual condition, in which the tracheid bar spans only a portion of the ventral testa thickness. **k.** Ventral exterior view of hilum, showing two hilar tongues. Epitestal deposits surround hilum. **l.** Lateral exterior view of testa, showing clear, colorless, viscid epitestal deposits with embedded foreign matter. **m.** Ventral exterior view of hilum, showing two hilar tongues. Epitestal deposits surround hilum. **n.** Cotyledon areole on abaxial surface of dry cotyledon under lateral light. **o.** After abscission between seed and funiculus above counter-palisade, seed held in position near funiculus by two hilar tongues. Vascular bundle from funiculus extends out of hilum; it more commonly breaks off at hilum surface.

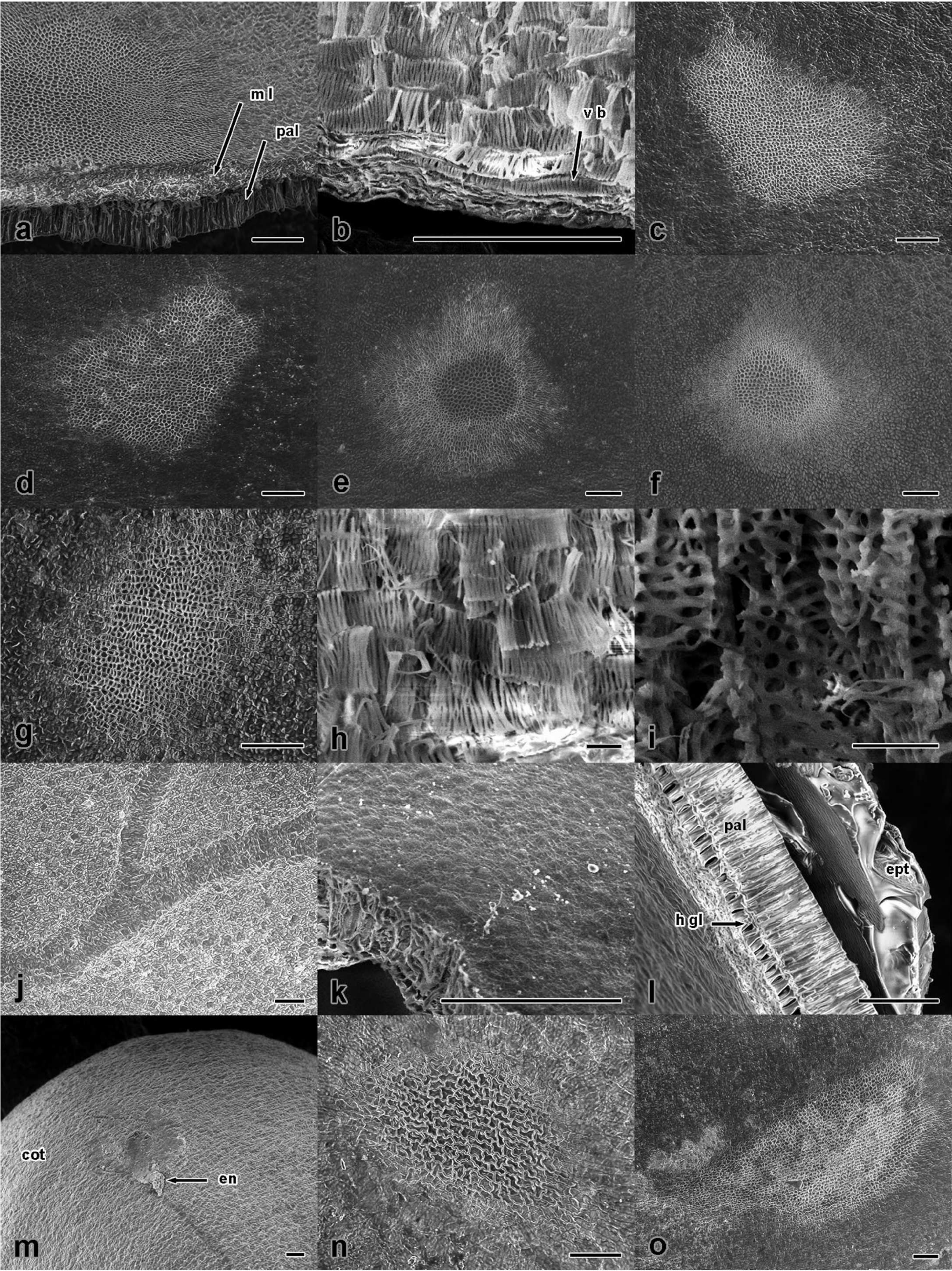


FIG. 3. ESEM of Clitoriinae seeds. a–b. *Centrosema arenarium* JAL 1262. c–d. *Centrosema plumieri* JAL 1266. e–f. *Centrosema virginianum* JAL 203. g–i. *Periandra heterophylla* JAL 252. j–k. *Clitoria amazonum* JAL 1594. l. *Clitoria laurifolia* JAL 1594. m. *Clitoria rubiginosa* JAL 295. n. *Clitoria termatea* JAL 197. o. *Barbieria pinnata* JAL 1271. Scale bars are 10 μm in h–i, 100 μm all others. a. Inner testa surface, showing cotyledon areole impression into endosperm and enlarged middle endosperm layer. b. Longitudinal section of lens cells. Tracheoids external to raphe vascular bundle. c. Abaxial

Embryo consists of two cotyledons, radicle, and large plumule between cotyledons. Radicle oriented towards micropyle. Cotyledon areole ovate, medial, ca 0.3×0.3 (Fig. 3e), and immediately internal to lateral endosperm thickening (Fig. 3f), stains about equally as surrounding cells with toluidine blue; imbibition damage of surrounding cells severe. Cells arranged more regularly in cotyledon areole than surrounding epidermal cells.

Centrosema arenarium. Seed as in *Centrosema virginianum*. Seed spherical to cylindrical; ca 5.5×4.5×5.5 mm; brown with brown hilum. Hilar tongue minute or wanting. Micropyle oval. Counter palisade covered by material that appears to be fungal hyphae. Lens tracheoid cells (Fig. 3b) contiguous with tracheid bar by attenuated white tissue. Cotyledon areole (Fig. 3a) ovate, medial to medial-apical, ca 0.5×0.8.

Centrosema brasilianum. Seed as in *Centrosema virginianum*. Seed reniform-cylindrical; ca 4.0×2.2×2.8 mm; brown to brown-tan mottled with brown hilum. Micropyle Y-shaped to broadly oval. Lateral testa exterior usually conspicuously swollen at site of endosperm deposits (Fig. 2b). Cotyledon areole circular, medial, ca 0.2×0.2.

Centrosema molle. Seed as in *Centrosema virginianum*. Seed reniform-cylindrical; ca 5.0×2.7×3.2 mm; brown or brown-tan mottled with brown hilum. Micropyle Y-shaped to broadly oval. Cotyledon areole broadly ovate, medial, ca 0.2×0.2.

Centrosema pascuorum. Seed as in *Centrosema virginianum*. Seed reniform-cylindrical; ca 4.0×1.5×2.5 mm; brown with brown hilum. Micropyle Y-shaped to broadly oval. Lateral testa exterior sometimes slightly swollen at site of endosperm deposits; interior cotyledon areole impression prominent (Fig. 2c). Cotyledon areole broadly ovate, medial, ca 0.2×0.2.

Centrosema plumieri. Seed as in *Centrosema virginianum*. Seed spherical compressed; ca 7.0×5.0×6.0 mm; brown with brown hilum. Hilar tongue one-fourth length of hilum. Micropyle Y-shaped to broadly oval. Cotyledon mesophyll mostly spongy, one palisade-like layer on adaxial face. Cotyledon areole circular, medial-apical, ca 0.5×0.5 (Fig. 3c,d).

Centrosema sagittatum. Seed as in *Centrosema virginianum*. Seed reniform-cylindrical; ca 6.5×3.0×3.7 mm; brown with brown hilum. Micropyle broadly oval. Cotyledon areole broadly ovate, medial, ca 0.7×0.5.

Periandra densiflora. Seed as in *Centrosema virginianum*. Seed reniform-compressed; ca 3.5×?×3.5 mm (seeds immature, width uncertain); brown with brown hilum. Seeds not dissected, anatomy unknown.

Periandra heterophylla. Seed as in *Centrosema virginianum*. Seed reniform compressed, ca 4.8×1.9×2.9 mm, brown or brown-tan mottled (Fig. 1b). Funicular remnants on counter-palisade sparse to common, especially visible immediately inside the rim-aril. Lens cells (Fig. 3h) with scalariform thickenings, reminiscent of tracheid bar (Fig. 2f, 3i), which has reticulate thickenings or pits. Cotyledon mesophyll mostly spongy, 1–2 palisade like layers on adaxial face, several irregular palisade-like layers on abaxial face (Fig. 2h). Starch test negative or faint. Cotyledon areole circular, basal-medial, ca 0.2×0.2 (Fig. 3g); almost imperceptible under 50×. Plumule small to medium.

Periandra mediterranea. Seed as in *Centrosema virginianum*. Seed reniform compressed, ca 5.8×1.7×4.3 mm, brown with tan hilum. Funicular remnants on counter-palisade sparse. Cotyledon mesophyll spongy. Starch test negative. Lens tracheoid tissue poorly developed, 0.05 mm thick. Cotyledon areole ovate, medial, ca 0.4×0.2; almost imperceptible under 50×. Plumule small.

Clitoria (Clitoria) ternatea. Seed reniform to cylindrical; ca 5.6×2.9×4.2 mm; brown or brown-black mottled with brown hilum; sink in water (Fig. 1c).

cotyledon surface with cotyledon areole and surrounding cells. d. Inside endosperm surface from specimen in (c) with mirror image impression of cotyledon areole. e. Abaxial cotyledon surface with cotyledon areole and surrounding cells. f. Inside endosperm surface from specimen in (e) with mirror image impression of cotyledon areole. g. Abaxial cotyledon surface with cotyledon areole and surrounding cells. h. Longitudinal section of lens. i. Longitudinal section of tracheid bar. j. Abaxial cotyledon surface with impression of testa veins. k. Testa, showing that the surface is not smooth. l. Transverse section through testa and epitesta. Epitesta has fractured in curved planes and peeled from testa, showing testa impression on inner surface. m. Abaxial cotyledon surface with patch of endosperm remnants. n. Abaxial cotyledon surface with cotyledon areole. o. Inside endosperm surface with impression of cotyledon areole.

Seed externally with prominent hilum, anterior micropyle and posterior lens. Hilum oval ca 0.8×1.2 mm; surrounded by a slightly raised rim. Rim-aril tan, mostly of ca 0.2 mm raised bead, notched anterior; with hilar tongue ca 0.9 mm wide by 0.8 mm long attached along entire lateral side of hilum and distal end extended anterior. Hilar tongue on left in about half the seeds; on right in about half the seeds. Counter-palisade surface covered with white cellular funicular remnants, especially adjacent to the rim-aril. Vascular bundle scar between posterior end of hilar groove and rim-aril; white; raised; prominent. Hilar groove visible; when open, allows view of white tracheid bar below. Micropyle outside rim-aril, ca 0.2 mm long; Y-shaped when dry, with arms of Y towards hilum. Lens slightly perceptible as ca 0.4 mm raised area posterior to hilum. Epitesta deposits wanting.

Seed in transverse section through the middle of the hilum with testa, endosperm, and two cotyledons of the embryo. Testa 0.1 mm thick dorsally to 0.4 mm thick at the hilum. Palisade (Malpighian) layer of one cell-layer thick; thinner dorsally; light line immediately under the outer surface except at the hilum where it shifts inward. Hourglass layer immediately interior; of a single cell-layer; imperceptible dorsally and laterally, thicker ventrally, disappearing under the hilum. Parenchyma layer imperceptible dorsally and laterally with 50× optics; increasingly thicker on ventral half. Hilum testa much thicker and more differentiated than dorsal and lateral testa. Rim-aril and extension into hilar tongue top outside edge of counter-palisade and hilar rim. Funicular cell remnants cover counter-palisade, absent from hilar groove area. Hilum palisade and counter-palisade curved upwards towards hilar groove. Light line in hilum palisade about one-tenth down from outer surface; touching each other at the lips of the hilar groove when groove closed. Tracheid bar white; ovate; immediately below hilar groove; sometimes minutely cleft ventrally; no dorsal extension. Sclerenchyma below hilum dark. Parenchyma below hilar rim white, with columns of loosely joined cells with large air spaces. Endosperm thin over entire inside of testa; laterally forming two slightly thicker areas adjacent to cotyledon areoles. Cotyledons tan-yellow; adaxial mesophyll of palisade parenchyma; abaxial mesophyll slightly palisade-like; interior mesophyll spongy; starch test negative; vascular bundles about 5, central one slightly larger.

Seed in longitudinal section reveals the same basic testa, endosperm, and embryo structure. Testa palisade tissue thinner in lens area. Vascular bundle from funiculus joins the posterior end of the tracheid bar and extends into the raphe vascular bundle. Lens cells between Malpighian layer and raphe vascular bundle dark or light vertical cells. Endosperm adnate to and lines the entire inner surface of the testa; slightly transparent and dark, allowing partially obscured view of inner surface of testa through endosperm; when dry forms an impression of every feature of the embryo, down to the cellular level; thicker areas especially between radicle and cotyledon, and in a lateral thickening both left and right; cotyledon areole impression from embryo on each lateral endosperm thickening; swells on addition of water, especially hot water, becoming gelatinous, and embryo impression structure lost.

Embryo consists of two cotyledons, radicle, and minute plumule between cotyledons. Radicle oriented towards micropyle. Cotyledon areole ovate, medial to basal-medial, ca 0.9×0.4, and immediately internal to lateral endosperm thickening, stains less readily than surrounding cells with toluidine blue. Cells arranged more regularly in cotyledon areole than surrounding epidermal cells (Fig. 3n).

Clitoria (Neurocarpum) rubiginosa. Seed globose; ca 4.0×4.0×4.0 mm; brown with tan and brown hilum (Fig. 1e); epitesta irregularly covers most of seed; sink when forced under water.

Seed externally with prominent hilum, anterior micropyle and posterior lens. Hilum oval ca 0.9×1.1 mm; surrounded by a raised rim. Rim-aril tan, mostly of ca 0.1 mm raised bead; with hilar tongue ca 0.5 mm wide at base by 0.8 mm long positioned from slightly anterior of mid-hilum extending toward posterior end; a similar tongue, about half as wide and quarter as long, on opposite side of hilar tongue on posterior quarter. Larger hilar tongue on left in about half the seeds; on right in about half the seeds. Counter-palisade surface covered with white cellular funicular remnants. Vascular bundle scar between posterior end of hilar groove and rim-aril; white; raised; prominent. Hilar groove visible. Air bubble forms above counter-palisade and below hilar tongue upon immersion. Micropyle outside rim-aril, ca 0.1 mm long; Y-shaped when dry,

with arms of Y towards hilum. Lens slightly perceptible as furrow immediately posterior to hilum. Epitesta deposits covering most testa surface except in hilum, lens, and micropyle area, for seeds in pods or freshly removed from pods; embedded with endocarp cell remnants, extraneous plant parts and foreign material, and sporadic epitesta deposits on hilum, lens, and micropyle area for seeds exposed to storage or handling; absolutely clear and colorless; of endocarp origin; strongly adhesive and plastic when warm, spreading and becoming a viscous liquid at ca 55° C; non-adhesive and firm, almost brittle, when cool; of strongly hydrophobic material, when immersed in water, large air bubbles form on surface.

Seed in transverse section through the middle of the hilum with testa, endosperm, and two cotyledons of the embryo. Testa 0.08 mm thick dorsally to 0.4 mm thick at the hilum. Palisade (Malpighian) layer of one cell-layer; thinner dorsally; light line immediately under the outer surface. Hourglass layer immediately interior; of a single cell-layer; imperceptible dorsally, thin laterally, thicker ventrally, disappearing under the hilum. Parenchyma layer imperceptible dorsally and ventrally with 50× optics. Hilum testa much thicker and more differentiated than dorsal and lateral testa. Rim-aril and extension into hilar tongue and second tongue top counter-palisade at outside edge and on hilar rim. Funicular cell remnants on counter-palisade. Hilum palisade and counter-palisade curved upwards towards hilar groove. Light line in hilum palisade about one-twentieth down from outer surface; touching each other at the lips of the hilar groove when groove closed. Tracheid bar white; ovate; immediately below hilar groove; sometimes minutely cleft ventrally; spanning most of the testa, with no dorsal extension, always with dark sclerenchyma between tracheid bar and endosperm. Cells below hilum of dark sclerenchyma. Endosperm lining testa, scarcely perceptible. Cotyledons white to yellow; mesophyll of spongy parenchyma; starch test positive; vascular bundles 5, faintly perceptible, middle one largest.

Seed in longitudinal section reveals the same basic testa, endosperm, and embryo structure. Testa palisade tissue about equal thickness throughout. Vascular bundle from funiculus joins the posterior end of the tracheid bar and extends into the raphe vascular bundle. Lens tissue between raphe vascular bundle and Malpighian layer of dark vertically oriented sclerenchyma. Endosperm adnate to and lines the entire inner surface of the testa; thin and transparent, view of inner surface of testa through endosperm; thicker areas especially in slit between radicle and cotyledons, and as a ridge formed at juncture of cotyledon margins.

Embryo consists of two cotyledons, radicle, and minute plumule between cotyledons. Radicle oriented towards micropyle. Cotyledon areole wanting; small medial patch of endosperm often adherent to cotyledon (Fig. 3m). A patch of light-colored epidermal cells apically.

Clitoria (Neurocarpum) laurifolia. Seed as in *Clitoria rubiginosa*. Seed subglobose; ca 4.0×4.0×4.7 mm; brown with tan hilum; epitesta irregularly covers most of seed. Cotyledon areole wanting.

Clitoria (Neurocarpum) mariana. Seed as in *Clitoria rubiginosa*. Seed subglobose; ca 4.4×3.8×4.0 mm; brown to brown-black with tan hilum; thin epitesta irregularly covers most of seed. Second hilar tongue small to scarcely perceptible. Small patch of endosperm adjacent to cotyledon areole. Starch absent. Cotyledon areole circular, basal-medial, ca 0.3×0.3. Cotyledon mesophyll spongy, perhaps slightly palisade-like adaxially.

Clitoria (Bractearia) amazonum. Seed as in *Clitoria rubiginosa*. Seed circular, compressed, ca 7.4×3.4×7.5 mm; dark brown with tan hilum (Fig. 1d). Epitesta wanting (Fig. 3k). Hilar tongue attached to entire side of hilum. Second tongue smaller, attached on posterior hilum half (Fig. 2j). Testa venation massive (Fig. 2i); forms impression on cotyledons (Fig. 3j). Starch test positive. Cotyledon areole absent. Plumule unknown because of inadequate specimen.

Clitoria (Bractearia) brachycalyx. Seed as in *Clitoria rubiginosa*. Seed circular, compressed, ca 11.4×2.8×11.2 mm; dark brown with tan hilum. Epitesta wanting. Hilar tongue attached to entire side of hilum. Second tongue about a third as long, attached on entire opposite side. Testa covered with minute punctae. Testa venation massive; forms impression on cotyledons. Starch and cotyledon mesophyll unknown because of inadequate specimen. Cotyledon areole absent. Plumule unknown because of inadequate specimen.

Barbieria pinnata. Seed reniform-oval to cylindrical; ca 5.0×1.8×3.0 mm; brown with brown hilum; epitesta irregularly covers most of seed; sink when forced under water (Fig. 1f).

Seed externally with prominent hilum, anterior micropyle and posterior lens. Hilum oval ca 0.7×1.5 mm; surrounded by a raised rim. Rim-aril tan, mostly of ca 0.1 mm raised bead; with hilar tongue ca 0.7 mm wide by 0.6 mm long positioned from mid-hilum extending toward anterior end; a similar tongue, about equally wide and half as long, on opposite side of hilar tongue and slightly posterior. Hilar tongue on left in about half the seeds; on right in about half the seeds. Counter-palisade surface mostly naked; covered with white cellular funicular remnants adjacent to the rim-aril. Vascular bundle scar between posterior end of hilar groove and rim-aril; white; raised; prominent. Hilar groove visible; when open, allows view of white tracheid bar below; air bubble often forms on hilum upon immersion in water. Micropyle outside rim-aril, ca 0.05 mm long; Y-shaped when dry, with arms of Y towards hilum. Lens slightly perceptible as ca 0.4 mm irregular crack; 0.4 mm posterior to hilum. Epitesta deposits covering most testa surface except in hilum, lens, and micropyle area, for seeds in pods or freshly removed from pods; embedded with extraneous plant parts and foreign material, and sporadic epitesta deposits on hilum, lens, and micropyle area for seeds exposed to storage or handling; absolutely clear and colorless; of endocarp origin; absent from immature seeds and some other seeds and some specimens; strongly adhesive and plastic when warm; non-adhesive and firm when cool; of strongly hydrophobic material, when immersed in water, large air bubbles form on surface.

Seed in transverse section through the middle of the hilum with testa, endosperm, and two cotyledons of the embryo. Testa 0.08 mm thick dorsally to 0.5 mm thick at the hilum. Palisade (Malpighian) layer of one cell layer; thinner dorsally; light line immediately under the outer surface except at the hilum where it shifts inward. Hourglass layer immediately interior; of a single cell-layer; imperceptible dorsally, thin laterally, thicker ventrally, disappearing under the hilum. Parenchyma layer imperceptible dorsally and laterally with $50\times$ optics. Hilum testa much thicker and more differentiated than dorsal and lateral testa. Rim-aril and extension into hilar tongue and second tongue top counter-palisade at outside edge. Funicular cell remnants few; adjacent to rim-aril on counter-palisade. Hilum palisade and counter-palisade curved upwards towards hilar groove. Light line in hilum palisade about one-tenth down from outer surface; touching each other at the lips of the hilar groove when groove closed. Tracheid bar white; ovate; immediately below hilar groove; sometimes minutely cleft ventrally; with no dorsal extension. Parenchyma below hilum of columns of loosely joined dark cells with large air spaces. Endosperm dorsally and ventrally thin; laterally forming two thick pockets 0.07×1.3 mm, about equal to the thickness of the testa, aleurone layer imperceptible, middle and inner layers tan, vitreous. Cotyledons tan-yellow; concave on part of abaxial face to match expanded endosperm; mesophyll of palisade parenchyma; starch test negative; vascular bundles not seen.

Seed in longitudinal section reveals the same basic testa, endosperm, and embryo structure. Testa palisade tissue about equal thickness throughout. Vascular bundle from funiculus joins the posterior end of the tracheid bar and extends into the raphe vascular bundle. Lens tissue between raphe vascular bundle and Malpighian layer of dark sclerenchyma. Endosperm adnate to and lines the entire inner surface of the testa; slightly transparent and dark, allowing partially obscured view of inner surface of testa through endosperm; when dry forms an impression of every feature of the embryo, down to the cellular level; thicker areas especially between radicle and cotyledon, and in a lateral thickening both left and right; cotyledon areole impression from embryo on each lateral endosperm thickening; swells on addition of water.

Embryo consists of two cotyledons, radicle, and minute plumule between cotyledons. Radicle oriented towards micropyle. Cotyledon areole ovate, medial to basal-medial, ca 0.9×0.4 , and immediately internal to lateral endosperm thickening, stains less readily than surrounding cells with toluidine blue. Cells arranged more regularly in cotyledon areole than surrounding epidermal cells.

DISCUSSION

Clitoriinae seeds show a diversity of seed characters.

Cotyledon areole presence

All studied specimens have cotyledon areoles except *Clitoria* subgenus *Bractearia* and two species of *Clitoria* subgenus *Neurocarpum*. The current study gives first reports of cotyledon areole presence in the genera

Periandra and *Barbieria* as well as several named species of *Centrosema*, *Clitoria* (*Neurocarpum*) *mariana*, and *Clitoria* (*Clitoria*) *ternatea*. Absence in *Clitoria* subgenus *Bractearia* and *C. laurifolia* and *C. rubiginosa* seems to be confirmed by diligent searches with light microscopy and SEM. In *C. rubiginosa*, small endosperm remnants were often found in a medial position on the cotyledon (Fig. 3m), which has been found to be an occasional indicator of cotyledon areole presence in some scant endosperm specimens of some tribe Phaseoleae (unpublished results); however several attempts did not show clear cotyledon areoles or any other clear indication of cotyledon areoles in *C. rubiginosa*. When present, cotyledon areoles were always circular to ovate and in a medial to basal-medial or slightly medial-apical position, above the midvein, and opposite a local enlargement of endosperm. Some cotyledon areoles were small, especially in *Periandra*, and difficult to detect initially. In *Barbieria*, cotyledon areoles were the largest in the subtribe and conspicuous (Fig. 2n). No seeds were available of *Clitoriopsis mollis*, the single species of the remaining genus in the subtribe.

General observations of cotyledon areole position and appearance in these plants are in accord with previous observations for other papilionoid legumes (Beck 1878; Dzikowski 1936, 1937; Miksche 1961; Yaklich et al. 1984, 1986, 1987, 1989, 1992, 1995, 1996, 1998; Baker & Minor 1987; Endo & Ohashi 1997, 1998a, 1998b, 1999a, 1999b; Matarese & Fasci 2002; Ma et al. 2004). The most immediately obvious indication of cotyledon areole presence is an area of epidermal cells with “granular projections” (Endo & Ohashi 1998a), in other words, projecting external cell walls. All cotyledon areoles were identifiable by these projections, and cotyledon areoles seen here matched the previously reported characteristics of an unbranched single spot on the abaxial surface of each cotyledon, above the midvein, opposite an enlarged middle endosperm layer which takes a mirror-image impression of the cotyledon areole (Fig. 3c-d, e-f), and with cells of different size and shape than other cotyledon epidermal cells. Endo and Ohashi (1999a) reported one *Clitoria* sp. specimen and one *Centrosema* sp. specimen with medial circular to oval cotyledon areoles. Their two observations are confirmed by material seen here.

Cotyledon areole correlation with other seed characters

Presence and position of cotyledon areoles is always associated with an enlarged area of middle endosperm. And the size of the cotyledon areole seems to be roughly correlated with the size of the middle endosperm layer. For instance, cotyledon areoles in most *Centrosema* and *Periandra* species are small, and so are the middle endosperm layers, which can sometimes be seen as a small swelling on the testa outside (Fig. 2b). *Barbieria*, and to a lesser degree, *Clitoria* (*Clitoria*) *ternatea*, have larger cotyledon areoles, and these are associated with larger, visible endosperm. In *Clitoria* subgenera *Neurocarpum* and *Bractearia* neither cotyledon areoles nor discernible endosperm is found, except for a small visible patch of endosperm and associated cotyledon areole in *Clitoria* (*Neurocarpum*) *mariana*; endosperm was also noted in this species by Pammel (1899).

In addition, absence of cotyledon starch is correlated with presence of cotyledon areoles and endosperm, with the realization that the starch test employed here is relatively crude. Nadelmann (1890) and Bailey (1971) also mention a negative correlation between cotyledon starch and endosperm galactomann reserves. No correlation could be found to exist between presence of canavanine, restricted to seeds of *Centrosema* (Lackey 1977a), and any other seed character observed here.

This study expands upon Smith's (1981, 1983) findings, supported by earlier Pammel (1899) findings, that *Clitoria* (*Clitoria*) *ternatea* fit Smith's form 1 and *Centrosema molle* (as *C. pubescens*) and *Clitoria* (*Neurocarpum*) *mariana* fit form 4 cotyledon and seed arrangement. Form 1 seeds, found in about 58% of legume species, have leaf-like photosynthetic cotyledons, adaxial palisade tissue, endosperm, and are epigeal. A remaining 30% (form 4) of legumes have fleshy, storage cotyledons, no adaxial palisade tissue, no obvious endosperm, and are either hypogeal or epigeal. The final 12% (forms 2 and 3) are intermediate or anomalous forms. In the current study, *C. ternatea* and *Barbieria* are shown to be form 1 seeds. Both have palisade mesophyll tissue, and conspicuous endosperm. *Clitoria* (*Clitoria*) *ternatea* is epigeal (Candolle 1825; Lubbock 1892; Baudet 1974; Yeh et al. 1987). Germination of *Barbieria* is unknown, but can be presumed to be epigeal because of the conspicuous palisade cotyledon mesophyll. *Clitoria* subgenus *Bractearia* and most subgenus *Neurocarpum* species do not have cotyledon palisade mesophyll, lack obvious endosperm, do have cotyledon

starch storage, are epigeal or hypogeal, and fit Smith's form 4 seeds. *Clitoria* (*Neurocarpum*) *mariana* seems to be an intermediate form: no true palisade mesophyll is formed, the cotyledons are thick storage structures, a small amount of endosperm is present, germination is epigeal (Holm 1891), and a starch test is negative. Although Smith considered *Centrosema molle* to be type 4, species of *Centrosema* and *Periandra* did show some tendency for palisade-like adaxial cotyledon mesophyll, had small amounts of endosperm, no obvious starch storage, and are epigeal or hypogeal. They also could therefore be considered intermediate forms. All of these structural adaptations and observations, endosperm, cotyledon starch, cotyledon mesophyll palisade tissue, and germination epigeal or hypogeal, seem to be various adaptations to germination strategies (Smith 1983). As suggested here by correlation in Clitoriinae, cotyledon areoles also seem part of this character group.

Systematic value of seed characters

The survey given here, although limited in number of species and intensity, suggests several other structures may be of taxonomic and morphological interest, and deserve more study.

Two characters distinguish species of *Centrosema* and *Periandra* from species of other genera. The first is the peculiar lens cell type (Fig. 2c, d, g, 3b, h) which forms a white tissue of cells similar to the distinctive papilionoid tracheid bar cells (Lersten 1982), but the lens cells have scalariform thickenings, and are oriented parallel to the testa surface as well as the immediately dorsal raphe vascular bundle; cells of the tracheid bar have pits or reticulate thickenings, and are oriented perpendicular to the testa surface. In other genera of Clitoriinae, the lens cells do not appear to consist of white tracheoids or have such thickenings, and are unexceptional relative to other papilionoid legumes. The second distinguishing character is a dorsal wing or mere extension of the tracheid bar which appears at low magnification to project the tracheid bar to span the entire testa thickness and contact, or almost contact, the innermost boundary of the testa. Some specimens or species in genera other than *Centrosema* and *Periandra* approach this tracheid bar condition; in *Clitoria* (*Neurocarpum*) *laurifolia* and *C. (Neurocarpum) rubiginosa* the tracheid bar spans most of the testa, but always there is a layer of dark testa cells between the tracheid bar and the innermost layer of testa.

A distinct cotyledon palisade mesophyll is found only in *Clitoria* subgenus *Clitoria* and in *Barbieria*. In these genera, about three prominent adaxial layers and several smaller abaxial layers surround a central spongy layer. Mesophyll entirely of spongy tissue was in *Clitoria* subgenus *Bractearia* and most *Clitoria* subgenus *Neurocarpum*. In *Centrosema* and *Periandra*, and possibly *Clitoria* (*Neurocarpum*) *mariana*, mesophyll was spongy and no true palisade tissue exists, but some mesophyll cells, particularly adaxial mesophyll cells, had one or two cell layers of somewhat palisade-like orientation (Fig. 2h). Current findings concur with Pammel's (1899) report of palisade mesophyll in *Clitoria* (*Clitoria*) *ternatea* and no palisade mesophyll in *C. mariana* and Smith's (1983) report of palisade tissue in *C. ternatea* and only spongy mesophyll in *Centrosema molle* (as *C. pubescens*).

In *Barbieria* and to a lesser degree in *Clitoria* subgenera *Neurocarpum* and *Bractearia*, the hilum bears the common papilionoid hilar tongue on the left or right, and a second smaller hilar tongue on the opposite side (Fig. 2k, m, o). In some specimens of *Clitoria* (*Neurocarpum*) *mariana* the second tongue is very small or essentially missing. Two hilar tongues are uncommon in other Phaseoleae (Fig. 2e). To my knowledge, it occurs conspicuously in the Phaseoleae only elsewhere in *Amphicarpaea*, *Dumasia*, *Cologania*, and *Dysolobium*; however, slight and barely perceptible suggestions of a second hilar tongue or minute hilar tongue pair (as in *Galactia* spp.) may occur in other genera (unpublished results). Berg (1979) described the development of the common single hilar tongue in Phaseoleae subtribe Kennediinae and the possible role in seed abscission. The function of a second hilar tongue is unknown.

An odd viscid thermoplastic epitesta occurs in *Barbieria* and *Clitoria* subgenus *Neurocarpum* (Fig. 2k, l, m, o, 3l). Noted at least since Bentham (1858) as a "peculiar viscid exudation" or "glanduloso-viscosa" in *Clitoria* subgenus *Neurocarpum*, in contrast to other species with "smooth" or "laevia" seeds. Fantz (1996b) and Kirkbride et al. (2003) additionally reported this epitesta in *Barbieria*. The term "smooth," as used here, is not appropriately the opposite of viscid. In Lersten's (1981) survey of legume testa patterns, the term smooth, or levigate, is one of nine testa patterns under SEM. Presence of epitestal deposits was considered a character distinct from these nine patterns. He reported testa patterns in *Centrosema*, *Clitoria*, and *Barbieria*

were rugulate to irregular papillose. None were considered smooth or levigate. The current study concurs with Lersten that the testa (Fig. 3k) is patterned, not smooth, under low magnification ESEM. It would, therefore, seem more appropriate to follow Lersten, and restrict the use of the term smooth for true testa patterns, and separately describe seeds as epitestal or non-epitestal.

The epitesta seen here, differs considerably from the epitesta common in Glycininae (Schleiden & Vogel 1839; Newell & Hymowitz 1978; Lackey 1981; Gijzen et al. 1999), even though both are of endocarp origin. The epitesta of Glycininae is dark, opaque, in cell-like or other rough irregular patterns, dusty or friable, and is unaffected by temperatures to about 60° C. Gijzen et al. (1999) showed that this epitesta in soybean was a protein, which, when sequenced, exhibits a short hydrophilic end and a longer hydrophobic end. The epitesta in *Barbieria* and *Clitoria* subgenus *Neurocarpum* is much different. As with Glycininae, it also covers most testa surfaces except the hilum, lens, and micropyle area for seeds in pods or freshly removed from pods, but it differs in spreading upon warm contact to other areas of the testa and other objects, and becomes embedded with extraneous plant parts and foreign matter. It is absent from some immature seeds and some other seeds and some specimens. It is absolutely clear and colorless, strongly adhesive and plastic when warm, and non-adhesive and firm, almost brittle, when cool. When these testa adherent deposits are cool and broken, they form curved fracture planes and peel away from the testa, exposing an inner epitestal surface with an impression of testa patterns (Fig. 3l). Above 55° C, the epitesta becomes a viscous, spreading, sticky liquid, which cools to a smooth, shiny, surface (Fig. 2m). It is of strongly hydrophobic material. When immersed in water, large air bubbles form on the surface. The functional significance of these deposits must await further study.

In *Clitoria* subgenus *Bractearia*, testa venation from the two recurrent vascular bundles is especially prominent (Fig. 2i), to a degree that impressions are formed on the abaxial cotyledon surface (Fig. 3j). No other specimens exhibited such a tendency. The functional need for such massive veins is unknown.

Generic circumscription (Fantz 1996b) in what is now considered Clitoriinae has remained reasonably stable since Bentham (1858, 1865), who recognized four genera: *Centrosema*, *Periandra*, *Clitoria*, and *Barbieria*. *Clitoria* was further divided into three sections. *Barbieria* was distanced from the remaining members and allied with *Tephrosia* (Bentham 1865). Wilczek (1954) added the monotypic genus *Clitoriopsis*. Recent taxonomic work, aside from the revisions of *Centrosema* (Barbosa-Ferevereiro 1977) and Brazilian *Periandra* (Funch & Barroso 1999), has been dominated by the works of Fantz (1976, 1979a, 1979b, 1988, 1991, 1996a, 1996b, 2000, 2001, et lit. infra), which focused on *Clitoria*, and presented the first serious study since Bentham (1858). Generic limits remained as with Bentham, with the addition of *Clitoriopsis*, acknowledgment of *Barbieria* as a distinct genus near *Clitoria*, and recognition of Bentham's *Clitoria* sections as subgenera.

The question of recognition of *Clitoria* as a single genus, or two or three genera, and the affinity of *Barbieria* with these has long been a source of ambiguity. Bentham (1858) merged *Neurocarpum*, which he formerly held to be generically distinct, with *Clitoria*, and formed another section, now known as subgenus *Bractearia*. Lackey (1977b) was inclined to divide *Clitoria* to the status before Bentham's merger. Fantz (1996a) recognized that one could argue for three distinct genera, but that the flowers are unique within the papilionoid legumes, and therefore maintained a single genus. *Barbieria* has, likewise, always caused much trouble. Originally described as a species of *Galactia* in 1807, of *Clitoria* in 1811, and then transferred to the new genus *Barbieria* in 1825 (Fantz 1996a), it had been aligned with several legume groups before realignment with and returned as a species of *Clitoria* (Lackey 1981). The realignment was suggested by Rob Geesink (personal correspondence 1979), who thought *Barbieria* better placed close to *Centrosema* or *Clitoria*. Fantz (1996a) used extensive morphological evidence to argue for a resegmentation of *Barbieria*.

Studies using molecular techniques do not clarify much at this time. Doyle and Doyle (1993), used cpDNA inverted repeat regions, to study phylogeny in 43 genera of tribe Phaseoleae. They only sampled *Centrosema virginianum* and *Clitoria* (*Clitoria*) *ternatea*, and found these two formed a close pair relative to other samples. Kajita et al. (2001), using rbcL sequences and the same two species, found essentially the same thing, but as part of a broader survey. The generic and subtribal structure has not been studied by molecular techniques.

Results of the current limited study of seeds, admittedly in need of much broader survey for taxonomic purposes, suggest a few affinities within the Clitoriinae.

Despite individual species variation, *Centrosema* and *Periandra* seeds are essentially all the same in basic form, and distinct from anything else in the subtribe. Aside from consistent medial cotyledon areoles with an adjacent patch of endosperm from an expanded middle endosperm layer (often externally visible as a lateral protrusion of the testa), there is the peculiar and consistent lens structure of white tracheoids. In addition, the tracheid bar extension to the full thickness of the testa sets these two genera apart.

The three subgenera of *Clitoria* have distinctive seeds. Subgenus *Clitoria*, only represented here by a single species, has cotyledon areoles, adjacent endosperm enlargement, palisade cotyledon mesophyll, negative starch test, inconspicuous testa venation, a single hilar tongue, and no epitesta. Subgenus *Bractearia* has no cotyledon areoles, no visible endosperm enlargement, only spongy cotyledon mesophyll, positive starch test, conspicuous testa venation, a second hilar tongue, and no epitesta. Subgenus *Neurocarpum* usually has no cotyledon areoles, no visible endosperm enlargement, only spongy cotyledon mesophyll, positive starch test, inconspicuous testa venation, a second hilar tongue, and the peculiar viscid thermoplastic epitesta. *Clitoria* (*Neurocarpum*) *mariana* does not fit the pattern for the remainder of the subgenus. It differs by presence of cotyledon areoles, endosperm presence, and negative starch test. It would be enlightening to see if a broader review would maintain these distinctions or clarify the relationships of the subgenera.

Barbieria has equally distinctive seeds. With *Clitoria* subgenus *Neurocarpum* it shares the peculiar epitesta and second hilar tongue, but almost no other seed characters, with the exception of *Clitoria* (*Neurocarpum*) *mariana*. *Barbieria* has prominent cotyledon areoles, conspicuous endosperm enlargement, prominent palisade cotyledon mesophyll, and a negative starch test. These characters place *Barbieria* in a peculiar position. If one regards the endospermic seed with palisade cotyledon mesophyll as basic and the non-endospermic seed with spongy cotyledon mesophyll as derived (Smith 1983), then *Clitoria* subgenus *Clitoria* and *Barbieria* are basic for this condition, and most members of the other subgenera of *Clitoria* are derived. On the other hand, one could assume that the peculiar epitesta is a derived character, and therefore unites *Clitoria* section *Neurocarpum* and *Barbieria*, even though they are currently placed in two distinct genera.

A good deal more information on the diverse seed anatomy of Clitoriinae is needed for further taxonomic interpretation.

ACKNOWLEDGMENTS

Thanks go to Joe Kirkbride for thoughtful discussion and use of facilities and collections at BARC. Scott Whittaker and Stanley Yankowski were most helpful with ESEM and anatomical work. John Kress and Warren Wagner graciously made available the collections and facilities of the Smithsonian Institution.

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